

UC Davis

UC Davis Previously Published Works

Title

Establishing What Constitutes a Healthy Human Gut Microbiome: State of the Science, Regulatory Considerations, and Future Directions.

Permalink

<https://escholarship.org/uc/item/9m94w9jp>

Journal

The Journal of nutrition, 149(11)

ISSN

0022-3166

Authors

McBurney, Michael I
Davis, Cindy
Fraser, Claire M
et al.

Publication Date

2019-11-01

DOI

10.1093/jn/nxz154

Peer reviewed

Establishing What Constitutes a Healthy Human Gut Microbiome: State of the Science, Regulatory Considerations, and Future Directions

Michael I McBurney,¹ Cindy Davis,² Claire M Fraser,³ Barbara O Schneeman,⁴ Curtis Huttenhower,⁵ Kristin Verbeke,⁶ Jens Walter,⁷ and Marie E Latulippe⁸

¹Human Health & Nutritional Sciences, University of Guelph, Guelph, Canada; ²National Institutes of Health, Bethesda, MD; ³Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD; ⁴Nutrition, University of California–Davis, Davis, CA; ⁵TH Chan School of Public Health, Harvard University, Boston, MA; ⁶Chronic Diseases, Metabolism & Ageing, KU Leuven, Leuven, Belgium; ⁷Agricultural, Food, & Nutritional Science, University of Alberta, Edmonton, Canada; and ⁸The International Life Sciences Institute, North American Branch, Washington, DC

ABSTRACT

On December 17, 2018, the North American branch of the International Life Sciences Institute (ILSI North America) convened a workshop “Can We Begin to Define a Healthy Gut Microbiome Through Quantifiable Characteristics?” with >40 invited academic, government, and industry experts in Washington, DC. The workshop objectives were to 1) develop a collective expert assessment of the state of the evidence on the human gut microbiome and associated human health benefits, 2) see if there was sufficient evidence to establish measurable gut microbiome characteristics that could serve as indicators of “health,” 3) identify short- and long-term research needs to fully characterize healthy gut microbiome–host relationships, and 4) publish the findings. Conclusions were as follows: 1) mechanistic links of specific changes in gut microbiome structure with function or markers of human health are not yet established; 2) it is not established if dysbiosis is a cause, consequence, or both of changes in human gut epithelial function and disease; 3) microbiome communities are highly individualized, show a high degree of interindividual variation to perturbation, and tend to be stable over years; 4) the complexity of microbiome–host interactions requires a comprehensive, multidisciplinary research agenda to elucidate relationships between gut microbiome and host health; 5) biomarkers and/or surrogate indicators of host function and pathogenic processes based on the microbiome need to be determined and validated, along with normal ranges, using approaches similar to those used to establish biomarkers and/or surrogate indicators based on host metabolic phenotypes; 6) future studies measuring responses to an exposure or intervention need to combine validated microbiome-related biomarkers and/or surrogate indicators with multiomics characterization of the microbiome; and 7) because static genetic sampling misses important short- and long-term microbiome-related dynamic changes to host health, future studies must be powered to account for inter- and intraindividual variation and should use repeated measures within individuals. *J Nutr* 2019;149:1882–1895.

Keywords: microbiome, microbiota, prebiotic, probiotic, dietary fiber, biomarker, surrogate indicator, dysbiosis, human health

Introduction

Microbial colonization of the human body begins postpartum (1–4) and proceeds in an incremental manner from infancy to adulthood, with the largest microbial community being found in the distal regions of the adult human gastrointestinal tract (5). Intestinal colonization occurs during infancy (5–7) and is affected by mode of delivery (8, 9), diet (10–12), probiotic supplementation (13, 14), antibiotic use (15, 16), and possibly maternal microbiome during pregnancy (4, 17). The resilience of a microbiota—that is, the capacity

to return to an equilibrium state in response to chemical (e.g., diet, antibiotic), physical (e.g., changes in rate of intestinal passage, pH), or microbial (e.g., probiotic supplementation, fecal transplants) perturbations—is a metric that seems to be associated with higher microbial diversity (18). Numerous published reports have described associations between altered microbiota composition and various diseases; however, there have been few consistent changes in microbiota stability, resilience, or diversity associated with a given disease across multiple cohorts.

Elucidation of other characteristics of a microbiome predictive of health would provide a target for interventions and microbial modifications in generally healthy populations and individuals exhibiting disrupted microbiota and associated diseases (19). Validated indicators for which a scientific consensus could be achieved could contribute to a framework to guide policymakers and regulators on marketing and product claims pertaining to a “healthy human microbiome.” This article summarizes the state of the science pertaining to the human gut microbiome and associated health benefits and identifies research gaps and opportunities.

State of the Evidence

In 2012, the International Life Sciences Institute (ILSI) North American Microbiome Committee commissioned a review on the question “what constitutes a healthy human gut microbiome” (19) that came to the following conclusions: 1) a healthy microbiome cannot be defined by a single idealized community composition, 2) a healthy microbiome is more resistant and resilient to disruption, 3) certain microbial distributions may increase susceptibility to infection and disease, and 4) it is unknown if dysbiosis, an imbalance in the types of microorganisms present in a given microbiota, is a cause or consequence of disease. For this article, we revisited the central question and evaluated whether the state of the evidence has changed substantially in the past 6 y.

Biological Considerations in Defining a Healthy Gut Microbiome

The gut microbiota clearly influences the health of its host. It provides crucial benefits in the form of immune system development, prevention of infections, nutrient acquisition, and perhaps even brain and nervous system functionality (20). In addition, the microbiome does clearly play a causal role in the development of pathologies in animal models of human disease, such as obesity (and associated pathologies), autoimmune diseases, and neurological diseases (21–27). In humans, causality is much harder to establish, but environmental factors known to disrupt microbiome assembly [e.g., route of birth delivery (8, 9, 28), formula vs. breastfeeding (28, 29), and early antibiotics (30)] are linked with disease risk in epidemiological studies (31, 32). In addition, human diseases are often associated

with a “dysbiosis” of the gut microbiota, meaning an altered composition or functionality compared with healthy controls. However, dysbiotic patterns are context and disease specific and often not consistently detected among different studies. In addition, for most diseases, it is unclear if dysbioses are the cause or the consequence of disease, and molecular mechanisms by which altered microbiomes cause disease are lacking (27). Due to these complications, it is extremely difficult to define what constitutes a healthy microbiome. Below we discuss the ecological characteristics of the gut microbiome in early life and in adults, as well as the associations that have been associated with health.

Infant Microbiome Insights

The composition of the human gut microbiota changes dramatically during the first few years of life (6, 33) and then remains relatively stable (34). Microbial diversity in an infant gut increases over time (6, 8, 33), and the assembly process is affected by delivery mode, with the gut microbiota of infants born by caesarian section being less diverse during the first 2 y of life than those born vaginally (8, 9). Development of intestinal microbiota in infants is influenced by feeding method (breastfeeding vs. formula) (10, 12), use of probiotics and antibiotics (15, 30), and the introduction of complex dietary substrates during weaning (5, 35). Although these deterministic factors have a clear, measurable impact on microbiome composition, community assembly is also driven by stochastic (nonpredictable) ecological processes (36, 37). Experimental studies in a mouse model where host and environmental factors are strictly controlled find early-life colonization order influences the outcome of the community assembly through priority effects (38). Since the order of arrival of early colonizers is to a large degree random (e.g., in infants born by caesarian section) and largely influenced by life events such as antibiotics that can also not be predicted, stochastic ecological processes might account for the >70% of the interindividual variation of the human microbiome that is currently unexplained (39, 40).

Differences in the trajectory of early-life microbiome assembly during the first years of life may have long-term effects on not only microbiome ecology but also the development of host gastrointestinal and associated lymphatic tissues, which would determine the risk of immune-mediated diseases (9, 41). Early-life events, including mode of delivery and type of feeding, siblings, and sex, shape the developing gut microbiota (28). The early gut microbiota of breastfed, vaginal-born infants is dominated by *Bifidobacterium* and *Bacteroides* species, which have evolved with humans and have specialized in the utilization of human milk oligosaccharides (42). These genera could therefore be considered characteristic for a healthy infant microbiota, and they are greatly reduced through cesarean sections and formula feeding (8, 9). However, in the absence of clinical data, there is insufficient evidence to define a universal standard for intestinal colonization and development of the infant microbiome (5), and this report will focus on defining the role of a healthy gut microbiome in adults.

Adult Microbiome Insights

The gut microbiome is a remarkably stable microbial community in healthy adults composed of highly adapted microbial

Supported by ILSI North America.

Conflict of interest and funding disclosure: MIM is a self-employed consultant/scientific advisor to agrifood, dietary supplement, and nutritional diagnostic device industries; adjunct professor at the University of Guelph; and owns stock in DSM, a B2B manufacturer of vitamins, carotenoids, and ω -3 fatty acids for use in human and animal products. MIM received funding to write the manuscript. CH has received consulting fees from Seres Therapeutics, a company that develops microbiome therapeutics, and Microbiome Insights, a microbiome data generation company, and serves on their respective scientific advisory boards. JW has received research funding and consulting fees from industry sources involved in the manufacture and marketing of dietary fibers and probiotics and is a co-owner of Synbiotics Solutions, a developer of symbiotic products. MIM, BOS, CH, KV, and JW received travel funding to attend the workshop. CD, CMF, and MEL have no conflicts of interest or funding to disclose. Address correspondence to MIM (e-mail: McBurney23@gmail.com).

Abbreviations used: CHO, carbohydrate; EU, European Union; FMT, fecal microbiota transplantation; HELIUS, Healthy Life in an Urban Setting; ILSI, International Life Sciences Institute; LGG, *Lactobacillus rhamnosus* GG; NRV, nutrient reference value; SES, socioeconomic status.

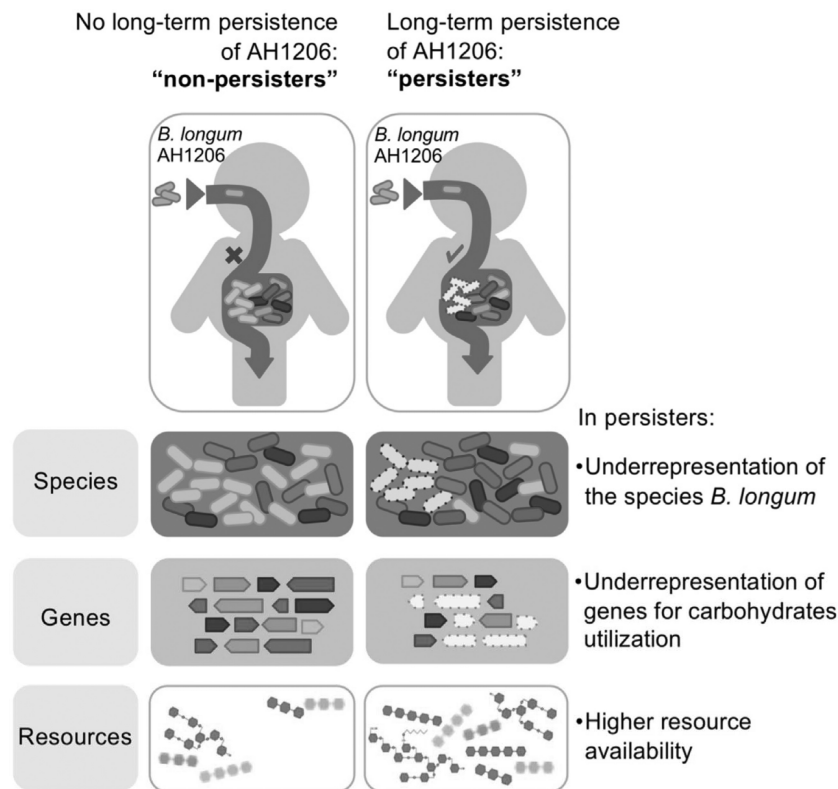


FIGURE 1 Long-term persistence or nonpersistence of an orally administered bacterial strain, AH1206, in 2 sets of individuals. Reproduced with permission from Maldonado-Gómez et al. (67).

species (43, 44) and shaped more by environment than host genetics (39, 45). Nongenetic and genetic factors each account for ~10% of the variation in gut microbiota, whereas effects of specific environmental factors (diet, medication, specific genes, etc.) account for ~20% (39, 40), making the majority of interindividual variation in the human gut microbiota unexplained. Ecological theory predicts that such unpredictable variation is driven by stochastic elements in the ecological processes that shape ecosystems (38).

High microbiota diversity seems to be associated with health and temporal stability (33, 34), and a dynamic loss of diversity may be prognostic of increased disease risk (34, 46). A physically inactive lifestyle and the consumption of a diet that is high in refined carbohydrate and salt and low in dietary fiber (47, 48) are associated with a depleted microbiome and increased prevalence of chronic disease linked to the gut microbiome (49). When confronted with environmental perturbations—for example, dietary interventions [isocaloric diets differing in fat and carbohydrate content (50) or 4 probiotic multispecies provided at breakfast in a capsule, low-lactose yogurt, or low-fat semihard cheese (51)] or antibiotics [500 mg ciprofloxacin twice a day (52)]—the gut microbiome changes (50–52). Overall, the composition of the diet influences the metabolic output of the microbiota (50), with unhealthier, Western-style diets rich in saturated fat and meat and low in fiber, leading to metabolite profiles likely to be detrimental to health (50, 53). Shifts in the composition and metabolic signatures of an individual's gut microbiome are seen in response to acute dietary [e.g., addition of resistant starch or fruits, vegetables, and legumes (54–56)] and medical interventions [e.g., antibiotics (52)]. Microbial diversity is negatively correlated with stool consistency and stool frequency, and stool consistency was

shown to make the largest contribution to interindividual fecal microbiota variation, although its overall contribution to the total variation is still small (57, 58). Longer colonic transit times have been positively correlated with gut microbiota diversity and richness, a shift in microbial metabolism from carbohydrate fermentation to protein catabolism, and higher urinary levels of potentially deleterious protein-derived compounds (59). If perturbations are a consequence of dietary change (60–62), the microbiome essentially reverts to its initial composition due to its resilience (56). If more severe (e.g., through repeated courses of antibiotics), the repair of the microbiome is incomplete, and members are lost (52, 63, 64).

The gut microbiome is altered in various diseases, a state often referred to as dysbiosis (65). Redressing such alterations through strategies such as fecal microbiota transplantation (FMT), probiotics, or live biotherapeutics may shape the development and function of the gut, its associated immune system, and other organs. Establishing persistent long-term changes in taxonomic structure to affect the long-term health of the host may be possible with FMTs and probiotics (66, 67) (Figure 1). However, a substantial knowledge gap exists about what constitutes a healthy microbiome and if such a microbiome can even be defined, as a definition may be both subject and context (e.g., disease) dependent. Most important, it is unclear if dysbioses are the cause of the disease or just a consequence (27).

There is evidence that the microbiome is affected by many characteristics of the host, including ethnicity, region of habitation, geography, and/or socioeconomic status (45, 46, 68, 69). Cross-population surveys find regional differences in susceptibility and prevalence of disease may be explained by differences in gastrointestinal microbiota (45). Individuals

living in neighborhoods with higher socioeconomic status (SES) exhibit indicators of greater microbiota diversity (vs. low SES) that do not appear to be a proxy for other demographic characteristics such as age, sex, self-reported racial/ethnic heritage, or lifestyle factors such as adiposity, smoking, or alcohol consumption (70). Unfortunately, alcohol intake (an exclusion criterion for the healthy controls) was the only dietary assessment in this study. The Healthy Life in an Urban Setting (HELIUS) study encompassed ~25,000 individuals from 6 ethnic groups living in Amsterdam, The Netherlands, who donated a stool sample (71). Within this single urban population, ethnic origin of participants was the strongest determinant of differences in fecal microbiota composition even after adjustment for dietary and nondietary factors (68). However, the lack of relationship between diet and microbiome may reflect the use of a single FFQ with 37 food groups being given to only 5 of 6 ethnic groups and then truncated into 4 dietary patterns for statistical analysis. It is possible that the HELIUS ethnic-specific FFQs were used (72), but distillation of diet composition to 4 patterns is not an accurate assessment. In a study of 1041 Israeli adults, the gut microbiota was more strongly influenced by environment than genetics, with environmental factors accounting for 20% of the total variance and these characteristics identified as sources of variance (percentage of total 20% variance): blood measurements (33%), vegetables (26%), meat products (16%), fruit (16%), and diet (5%). The dietary, ethnic, physical, and economic diversity of neighborhoods, urban or rural, makes the determination of factors causally affecting gut microbiome diversity and associations with human health elusive. Regional variation limits the development and application of healthy microbiome reference ranges (46).

Because of these challenges associated with identification and characterization of major gut microbiome patterns (73), enormous ecological gut microbial diversity among healthy adults (74), and ethnic origins and geographic locations (46, 68), it will likely prove extremely difficult to define a single, healthy microbiome.

Dietary Modulation

The health benefits of eating a diet rich in dietary fiber were recognized in 430 B.C. by Hippocrates (75). Except for its use as a natural remedy for constipation, dietary fiber was once considered a negative index of diet quality until low-fiber intake was associated with large bowel diseases (76). All dietary fiber definitions around the world include nondigested carbohydrate (CHO) and lignin inherent in food, and most include nondigestible CHO when extracted from edible material, synthesized, or modified if they have at least 1 demonstrated health benefit (77). It is now accepted that type, quality, and food origin shape the gut microbiome and microbiota-host interactions that may affect host health (78, 79).

A low dietary fiber intake does not support a healthy, diverse gut microbiome (80, 81) and is associated with degradation of the colonic mucus barrier on the gut epithelial lining (82, 83), as well as with production of nitrogen- and sulfur-containing compounds that are genotoxic and cytotoxic to colonocytes (59, 84–86). Ingestion of dietary fiber sources stimulates microbial proliferation, produces microbially derived end products [e.g., SCFAs and other microbial metabolites (81, 87)], and shifts the handling of colonic nitrogen by gut microbiota and host (59, 84, 88, 89). Twenty-four-hour urinary excretion of p-cresol, a

urinary toxin, is significantly decreased in healthy subjects after consuming 10 g of wheat bran daily for 3 wk (89) or 20–30 g lactulose daily for 4 wk (90).

A dietary prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (91, 92), although the requirement of “selectivity” has been questioned (93). The addition of a prebiotic to the diet may have health benefits; for example, galactooligosaccharide, a prebiotic, decreases intestinal permeability (94), improves laxation when dietary fiber intake is low (95), and may protect from low-fiber diet-induced colonic mucus deterioration (83, 96). Not all dietary fibers are considered prebiotic (97–99) and dietary fibers differ in their physicochemical impact within the colonic ecosystem (85, 100). The ingestion of some prebiotics, but not all, significantly increases absolute fecal numbers of *Bifidobacterium* and the percentage of total fecal microbiota (89, 101–103). It is very difficult to establish a causative role for the gut microbiome in the health effects of prebiotics (104), partly because of differences among individuals to prebiotic treatments in the microbiome ecosystem (94, 102) and heterogeneity in host functional outcomes that were measured [e.g., number of bowel movements per day (55, 89, 94, 95, 105)] and the possibility that health effects of dietary fibers and prebiotics are microbiome independent (106).

Probiotic Administration

A stable temporal core of species constitutes the majority (>75%) of the microbial community (73, 107), and these are not easily displaced (94, 108). In a study characterizing the effect of a *Lactobacillus rhamnosus* GG (LGG) intervention that achieved average fecal LGG counts 1000-fold higher than in the placebo group, the intervention was highly variable among individuals, did not alter the temporal stability of the microbiota, and did not correlate with changes in individual host functional measures [i.e., serum lipids (109)]. A systematic review of 7 randomized controlled trials testing the effects of administering probiotics to healthy adults did not find significant changes in fecal microbiota composition compared with control in any of the studies (110). Higher doses of multispecies probiotic formulations (70 billion CFU/d) seem to be necessary to achieve earlier, higher, and longer recovery in the feces (111). Persistence of probiotic strains in the human gut, in most studies, is temporal (only a few days) (112–115). When probiotics were administered as capsules, yogurt, or cheese, there was no effect on clinical chemistry (serum cholesterol, HDL, LDL, triglycerides, ALAT, ASAT, C-reactive protein, hemoglobin) or the composition of gut microbiota (51). However, one has to consider that probiotic strains are for the most part selected based on technological and not ecological criteria, and most probiotic strains belong to bacterial species that are not core members of the human microbiome (116). If an autochthonous core member of the gut microbiota is used (*Bifidobacterium longum* AH1206), stable persistence can be achieved for 6 mo after daily oral dosing but only in ~30% of individuals (67). Baseline resident fecal *B. longum* abundance was inversely associated with *B. longum* AH1206 persistence, possibly because indigenous organisms blocked the nutritional niche for the incoming probiotic (67). Thus, resident microorganisms of the gut microbiota may outcompete ingested probiotic strains (94). The lack of impact of probiotics on gut microbiota composition can be explained using ecological principles. The gut microbiota is

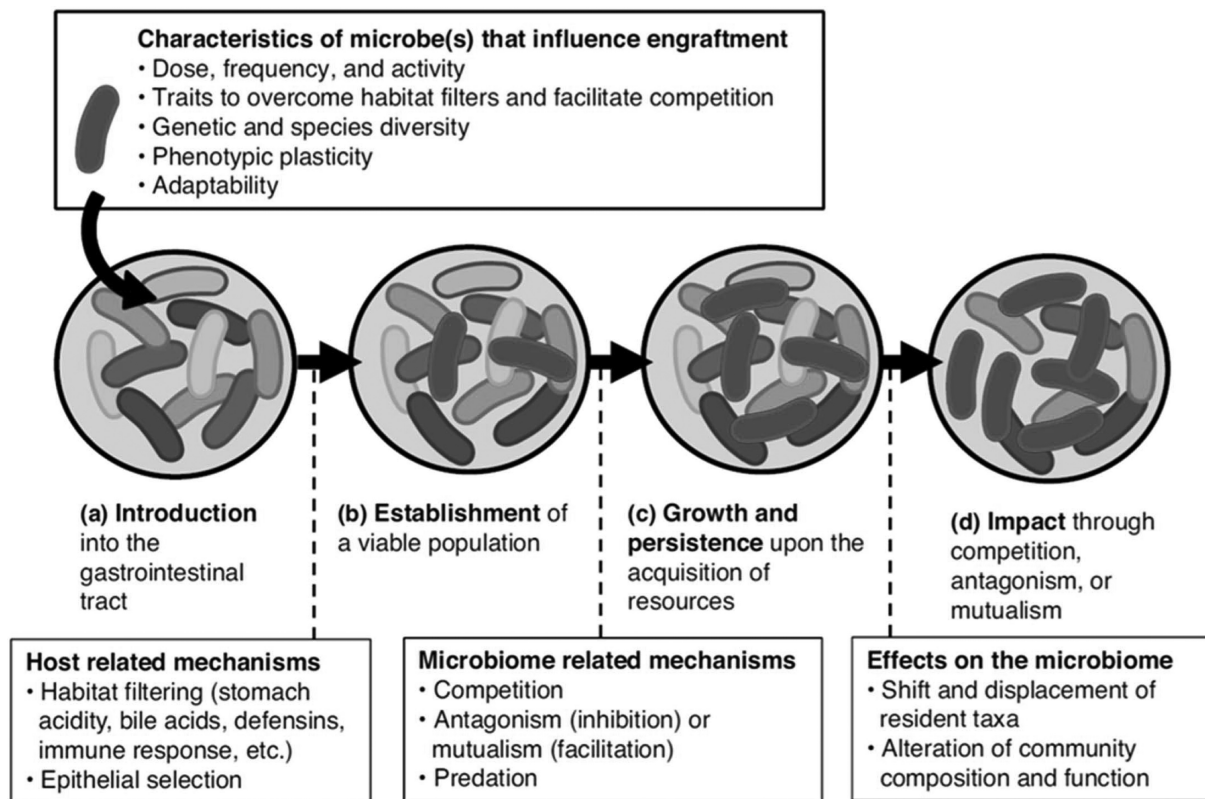


FIGURE 2 Successful invasion of an orally administered probiotic is a multistage process. Adapted with permission from Walter et al. (116).

highly resistant to incoming microbes, a trait referred to as “colonization resistance” (117). To become established, the incoming bacterial species must be able to overcome habitat filters in the gut and successfully compete for resources with the resident microbiota (116) (Figure 2). Even if engraftment is achieved, the probiotic strain has to engage in ecological interactions with the microbiota to alter the community. When studies with probiotics have not altered the gut microbiota, it might be due to a failure to consider ecological criteria in strain selection, production, and application, leading to strains being used that lack adaptations to the human gastrointestinal tract (116). However, it is important to consider that probiotics may improve health without changing the gut microbiota (e.g., by direct immunological, metabolic, and neurological effects on the host or by affecting the function of the commensal gut microbes). Consistent with this concept, data suggest that *LGG* may influence the function of other members of the gut microbiota (118). In this clinical trial in healthy adults, *LGG* did not alter the composition of the gut microbiota but did modulate its transcriptional profile in a subset of subjects. Expression of genes involved in flagellar motility, chemotaxis, and adhesion from *Bifidobacterium* and the dominant butyrate producers, *Roseburia* and *Eubacterium*, were increased during probiotic consumption in this cohort, suggesting that *LGG* may influence the activity of certain beneficial commensal bacteria and, thereby, indirectly affect host physiology.

Technical Challenges in Studying Gut Microbiome and Host Interactions

Measuring the human microbiome requires proper sample acquisition, handling, storage, and analysis. Procedures and

analytical methods need to be reproducible and consistently applied across studies and populations to obtain accurate diet and dietary intake assessments (119, 120) and to minimize technical variation in metagenomic data (73, 121, 122). For the most part, samples are obtained from defecated stools (122). Before genetic sequencing, each physical, chemical, and biological step involved in the molecular analysis of a microbial sample can be a source of bias, which could lead to a distorted perspective of the “real” microbiome (123). When extracting microbial nucleotides (DNA and/or RNA), microbial proteins, or microbial metabolites, each step can induce species-specific bias (124–126). DNA extraction is a common source of variation among laboratories (73, 127), partially because Gram-positive bacteria cell walls are more difficult to lyse.

As suggested (27), there is a need to focus on establishing causation and molecular mechanism with an emphasis on phenotypes that are large in magnitude, easy to measure, and unambiguously driven by the microbiota to ultimately identify the health and disease implications to humans. Such information is difficult if not impossible to obtain in sequencing surveys, which can only establish associations between microbiome features with host function or disease risk but not assign causation or directionality. Even if such associations are found, they are often not consistent among studies because of a lack of consistent sampling protocols and standards in microbiome research (46). It is currently difficult to integrate microbiome research findings into large databases in a meaningful manner because of the diversity of data and sample collection processes, sequencing, and bioinformatic protocols being used within research communities. There is a need for consensus and the adoption of standardized experimental and computational methods across studies and populations to minimize technical artifacts.

Regulatory Considerations with Respect to the Gut Microbiome

With an expanding body of microbiome-related research that is stimulating widespread interest in prebiotics and probiotics, regulatory agencies are increasingly confronted with proposals for health claims relating to the effects of diet on the gut microbiota. Permissible claims for the purpose of food labeling vary widely across the globe, although product labels and claims are expected everywhere to be accurate, truthful, and not misleading. The Codex Alimentarius Commission (Codex) publishes standards for voluntary adoption by any country. Codex has published general guidelines on nutrition labeling based on 2 types of nutrient reference values (NRV): 1) NRV-Requirements (NRV-R) based on levels of nutrients associated with nutrient requirements and 2) NRV-Noncommunicable Disease (NRV-NCD) based on levels of nutrients associated with reduction in the risk of diet-related noncommunicable diseases not including deficiency diseases or disorders (128). However, individual countries are left to develop guidance for specific foods and ingredients as they deem appropriate, including claims (129) that might relate to effects on the gut microbiome. The extent to which international regulatory bodies have addressed the latter varies widely.

Regulatory considerations in the United States, Canada, and Europe

In the United States, nutrition-related claims made in labeling are regulated through the Federal Food, Drug and Cosmetic Act, as amended (130). Within these regulations, nutrition-related claims consist of 4 types: 1) nutrient content claims, 2) structure-function claims, 3) health claims, and 4) dietary guidance claims (131–133). In Canada, nutrition-related claims consist of 1) nutrient content claims and 2) health claims, which are regulated through the Food and Drugs Act (134). In Europe, 1) nutrition and 2) health claims are options for food labeling (135).

Nutrient declarations

Nutrient declarations describe the amount of a nutrient in a food product, thereby characterizing the nutrient profile of a food. In these regions, regulations mandate the nutrients that must be presented on a label and the format of the label. Reference values for labeling purposes have been established by Codex (percent NRV), United States and Canada (percent Daily Value), and the European Union [EU; percent Reference Intake] and are usually based on authoritative evaluations of nutrient requirements for the generally health population (e.g., DRIs in the United States). In the United States and Canada, claims that a food is a good or excellent source of a nutrient require the establishment of a reference value for labeling purposes and are authorized through regulations (131, 132, 136). Similarly, the EU regulates nutrient claims and lists them in the annex of the Claims-Regulation (EU Directive 1924/2006).

In the United States and Canada, statements can be made for a dietary ingredient that does not have an established reference value (e.g., 240 mg DHA per serving, 12 mg lutein per serving, or 1 million CFU *Lactobacillus acidophilus*) per serving, but these statements must include the substance and the amount and not imply that the product is a good or excellent source of the substance (131, 136). The EU provides an option to make claims for “other nutrients” when added in a “significant amount,” a term that is not well defined by the regulation, and each proposed claim must be evaluated on a case-by-case basis.

For example, “containing *Lactobacillus acidophilus*” might be a possible claim in the EU.

Labeling regulations specific to some dietary fibers and prebiotics

Before a manufacturer can add and label an isolated or synthetic nondigestible carbohydrate as “dietary fiber” on a food product in the United States or Canada, US (137) and Canada (132) regulations require demonstration of “a physiological effect that is beneficial to human health.” These regulations are relevant when considering labeling a prebiotic. The FDA and Health Canada have published lists of dietary fibers that have been assessed and found acceptable as fiber sources (Table 1) (137, 138). If a synthetic or isolated nondigestible carbohydrate is not listed, a petition must be submitted requesting a review of the evidence of a physiological effect that is beneficial to human health. Examples of physiological effects that are beneficial to human health by the FDA and Health Canada are presented in Table 2 (139, 140). These lists do not include any reference to a “healthy gut microbiome.”

Structure-function and function claims

A structure-function (United States) or function (Canada and EU) claim is any statement linking the beneficial effects of a food or dietary ingredient with the normal functioning or biological activities of the body (e.g., dietary fiber to help maintain the health of the gastrointestinal tract). These claims cannot refer to a disease state or a reduction in disease risk (131, 141). In the United States and Canada, when requested by regulatory agencies, manufacturers must produce scientific substantiation describing the expected effect or benefit of the nutrient or ingredient and study design, and it must reflect the totality of the evidence (77, 142). In Europe, function claims must be authorized before they can be used (135).

Disease risk reduction claims

A disease risk reduction claim is any statement linking the consumption of a food or constituent of food with a reduction in risk of developing a diet-related disease or condition. Regulatory agencies in the United States, Canada, and Europe authorize disease risk reduction claims that are supported by significant scientific agreement (142–144). In Canada, premarket review and approval of claims about diseases or health conditions not listed in Schedule A to the Food and Drugs Act are voluntary (133).

To date, disease risk reduction claims have been authorized for fiber-containing foods and reduced risk of some types of cancer as well as foods that contain fiber (with emphasis on soluble fiber) from certain foods and reduced risk of coronary heart disease (144, 145). In 2009, Canada approved nonspecies-specific claims for probiotics in foods with a restricted list of species (146), but none relate to the gut microbiome. In the United States, guidance was issued on the types of studies from which the FDA can draw conclusions for evaluating health claims (147), but neither the FDA or Canada has approved any microbiome-specific claims for foods containing prebiotics. The EU-issued draft guidance specifically related to gut and immune function and the characterization of claims referencing a “beneficial physiological effect” and disease reduction (148).

Regulatory considerations in Asia and other regions

Claims related to the gut microbiome are more common outside of the United States, Canada, and Europe. In the Southeast Asia region, several examples of claims can be found (Table 3) (149–156).

TABLE 1 Lists of Isolated or Synthetic Nondigestible Carbohydrates Meeting the “Dietary Fiber” Definition in the United States and Canada

United States (137)	Canada (138)
<ul style="list-style-type: none">• β-glucan soluble fiber• Psyllium husk• Cellulose• Guar gum• Pectin• Locust bean gum• Hydroxymethylpropylcellulose<ul style="list-style-type: none">• Mixed plant cell wall fibers (a broad category that includes fibers like sugar cane fiber and apple fiber, among many others)• Arabinoxylan• Alginate• Inulin and inulin-type fructans• High-amylose starch (resistant starch 2)• Galactooligosaccharide• Polydextrose• Resistant maltodextrin/dextrin	<ul style="list-style-type: none">• Acacia gum or gum arabic• Barley β-glucan concentrate• Brans (barley, corn, oat, wheat)• Fructooligosaccharides or oligofructose• Galactooligosaccharides• Inulin from chicory root, Jerusalem artichoke tuber, blue agave head• Isomaltoligosaccharides• Oat β-glucan concentrate• Oat hull fiber• Partially hydrolyzed guar gum• Pea hull fiber• Peel (apple, blueberry, cranberry, orange)• Pulp (orange, tomato)• Polydextrose• Polysaccharide complex (glucomannan, xanthan gum, sodium alginate)• Maltodextrin• Resistant starches• Syrup (fiber)• Wheat flakes• Whole or edible parts of traditional fruits, vegetables, cereals, legumes, nuts, and seeds

Japan regulates health-related claims for food as “Food for Specified Health Uses” and “Food with Function Claims” (157). Health claims for gut microbiome can be declared under those regulatory systems with the required evidence support (150, 158, 159). Table 3 contains example claims from Japan and other Asian regions.

Brazil is developing a technical dossier for the approval of new probiotic products. As of February 2019, the draft concept is to include 2 types of claims. The first are *general claims*, which are specific to a “general function” benefit of the probiotic in the body. An example would be “better gastrointestinal health,” which could include outcomes such as improvements in intestinal transit time or reduced abdominal pain. The second are *specific claims*, which include benefits related to a physiologic or metabolic function. Studies supporting all types of proposed claims must be conducted in humans and may include systematic reviews, meta-analyses, randomized clinical studies, and prospective cohort studies. Benefits must be strain related (160).

What Type of Information Is Needed to Define a Healthy Gut Microbiome?

Despite the importance of the gut microbiota and diet-microbiome interactions for human health, North American and European regulatory agencies have not approved

microbiome-specific health claims. This is likely due to the inherent challenges and complexities that surround the definition of a healthy microbiome and a lack of validated biomarkers or surrogate end points to define and measure microbiome-host interactions. For regulatory agencies to evaluate and enforce claims related to the microbiome and its impact on host health, critical information is necessary that requires a comprehensive, multidisciplinary research agenda. In addition to existing data, prospective and cohort studies are needed to elucidate relationships between the gut microbiome (species and function) and biomarkers or surrogate end points in the host that are accepted indicators of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions (161), regardless of structure (gut epithelium, immune system, etc.) or biological sample (blood, feces, saliva, urine, etc.). Just as biomarkers have been identified and validated as surrogate end points for cardiovascular disease and diabetes (162), similar efforts are needed for the gut microbiome and host responses to the gut microbiome.

To identify such “microbiome biomarkers,” prospective studies are needed in well-characterized human populations to determine features (microbiota diversity, specific taxa, metabolic processes, gene clusters, metabolites, etc.) that predict disease risk or serve as surrogate markers of disease. Once such potential “biomarkers” have been identified, randomized, placebo-controlled clinical trials using a variety of microbiome-modulating strategies (probiotics, prebiotics,

TABLE 2 Examples from the United States and Canada of Physiological Effects of Nondigestible Carbohydrates That Are Considered Beneficial to Human Health

United States (139)	Canada (140)
<ul style="list-style-type: none">• Lowering blood glucose and cholesterol levels• Lowering blood pressure• Increase in frequency of bowel movements (improved laxation)• Increased mineral absorption in the intestinal tract• Reduced energy intake (e.g., due to the fiber promoting a feeling of fullness)	<ul style="list-style-type: none">• Improves laxation or regularity by increasing stool bulk• Reduces blood total and/or LDL cholesterol levels• Reduces postprandial blood glucose and/or insulin levels or increases sensitivity to insulin• Provides energy-yielding metabolites through colonic fermentation

TABLE 3 Approved Claims in Asia and Other Pacific Regions*Indonesia* (149)

- Soluble dietary fiber (psyllium, β -glucan from oats, inulin from chicory, and pectin from fruit) can help maintain/preserve the function of the digestive tract.

Japan (150–152)

- Bowel regulation by *Bifidobacterium longum* BB536
- lb81 lactic acid bacteria help balance the intestinal bacteria and keep the condition of the stomach maintained.
- Both galactooligosaccharides and dietary fiber work together to increase intestinal bifidobacteria and lactic acid bacteria and adjust the condition of the stomach.
- Live *Bifidobacterium* (*Bifidobacterium lactis* bb-12) help improves the intestinal environment and keep the condition of the stomach maintained.

Malaysia (154)

- Inulin and oligofructose (fructooligosaccharide):
 - Inulin helps increase intestinal bifidobacterial and helps maintain a good intestinal environment.
 - Oligofructose (fructooligosaccharide) helps increase intestinal bifidobacterial and helps maintain a good intestinal environment.
 - Inulin is bifidogenic.
 - Oligofructose (fructooligosaccharide) is bifidogenic.
 - Inulin is prebiotic.
 - Oligofructose (fructooligosaccharide) is prebiotic.
- High-amylose maize resistant starch helps improve/promote colonic/bowel/intestinal function/environment.
- *Bifidobacterium lactis* helps improve a beneficial intestinal microflora.
- Oligosaccharide mixture containing 90% (wt/wt) galactooligosaccharides and 10% (wt/wt) long-chain fructooligosaccharides:
 - The above oligosaccharide mixture is prebiotic.
 - The above oligosaccharide mixture is bifidogenic.
 - The above oligosaccharide mixture increases intestinal bifidobacteria and helps maintain a good intestinal environment.
- Polydextrose:
 - Polydextrose is bifidogenic.
 - Polydextrose helps increase intestinal bifidobacteria and helps maintain a good intestinal microflora.

Singapore (155)

- Inulin:
 - Inulin helps support growth of beneficial bacteria/good intestinal flora in gut.
 - Inulin helps increase intestinal bifidobacteria and helps maintain a good intestinal environment.
- Oligofructose stimulates the bifidobacteria, resulting in a significant increase of the beneficial bifidobacteria and the presence of less-desirable bacteria is reduced.
- Prebiotic promotes the growth of good *Bifidus* bacteria to help maintain a healthy digestive system.
- Probiotics
 - Probiotics help maintain a healthy digestive system.
 - Probiotics help in digestion.
 - Probiotics help to maintain a desirable balance of beneficial bacterial in the digestive system.
 - Probiotics help to suppress/fight harmful bacteria in the digestive system, thereby helping to maintain a healthy digestive system.

Thailand (154, 156)

- Gut health/function claims have not been approved.

synbiotics, dietary fiber, and fecal microbiota transplantation) should be tested to understand mechanistic relationships between the gut microbiome and host health (163). By combining multiomics characterization of microbiome with measures of host metabolome, associations can be elucidated that at least infer causation (which is extremely hard to establish). Both the identification of microbiome biomarkers and their validation are complicated due to the substantial variability of the microbial communities and the complex ecological factors that cause it. Studies therefore need to be well designed and conducted with adequate sample sizes among specific and well-defined populations to understand the roles of age, sex, life stage (pregnancy, lactating, menopausal stage), obesity, chronic disease, diet, human behavior (use of alcohol, antibiotics, tobacco), geography, environment (urban, rural), socioeconomic status, ethnicity, and pattern of immigration. Such studies should include detailed dietary data to help elucidate confounders, such as the impact of amount and

types of nondigestible carbohydrates consumed, local compared with nonlocal foods, organic compared with nonorganic foods, pesticide exposure, alcohol use, and water quality on the microbiome.

Consensus should also be achieved regarding mandatory baseline and follow-up data collection (biological samples, dietary data, health information, etc.), and processing of samples (collections, analysis, interpretation, and data analysis) should be standardized to understand microbiome-host relationships and allow microbiome studies to be compared and/or combined.

What Are Current Limitations to Establishing a Healthy Gut Microbiome-Host Relationship?

Researchers in the gut microbiome field are faced with a daunting complexity when trying to define a healthy gut microbiome

TABLE 4 Workshop Summary

- Causality has not been established between changes in gut microbiome structure and function and markers of human health.
- It is not established if dysbiosis is a cause, consequence, or both of changes in human gut epithelial function and disease.
- Microbiome communities are highly individualized, show a high degree of interindividual variation to perturbation, and tend to be stable over years.
- The complexity of microbiome-host interactions requires a comprehensive, multidisciplinary research agenda to elucidate relationships between gut microbiome and host health.
- Biomarkers and/or surrogate indicators of host function and pathogenic processes based on the microbiome need to be determined, along with normal ranges, and validated.
- Future studies measuring responses to an exposure or intervention need to combine validated microbiome-related biomarkers and surrogate indicators with multiomics characterization of the microbiome.
- Because of human gut microbiome dynamics, static genetic sampling misses important short- and long-term microbiome-related changes to host health, so future studies should be powered to account for inter- and intraindividual variation and should use repeated measures within individuals.

interrelationship. As an ecological community, the microbiome is structured by hundreds of genetic, environmental, and clinical factors and likely by stochastic ecological processes, driving vast interindividual variation and biogeographic differences in human populations (39, 40, 164). All this complicates the identification of microbiome features that determine health. In addition, microbiome features might influence risk of disease years if not decades before pathologies arise. Neither cause-and-effect relationships nor the molecular mechanisms are currently sufficiently understood (27). Without this information, it will be difficult to make progress on what constitutes a healthy gut microbiome and translate this information into tangible nutritional or pharmaceutical strategies and regulatory policies.

What Types of Studies Can Address These Gaps?

Because of the dynamics of the human gut microbiome, static genetic sampling misses important short-term and long-term microbiome-related changes to host health. Future studies need to be conducted in larger human populations to address interindividual variability and use repeated measures within individuals to help overcome intraindividual variability.

Multicenter comparative studies and workshops are needed to develop best practices for microbiome-host health studies. Research is needed to develop standards for the collection of dietary information, subject demographics, biological samples (timing, frequency, site), laboratory processing, genetic analyses, and data manipulation/analysis. Based on these findings, metabolomic indices in blood, feces, and urine and host-specific indicators of health should be identified, validated, and agreed on so that forthcoming consensus statements can be used to guide regulatory agencies. Large prospective and cohort studies measuring lifestyle (diet, culture, antibiotic use) effects in diverse populations (age, sex, life stage, disease, socioeconomic status, education, geography) on the microbiome, measuring both microbiome characteristics and metabolic functions of the microbiome and host and systematically linking them to disease risk, are needed to elucidate microbiome-host relationships. More hypothesis-driven clinical research is needed targeting microbiome traits to determine causality between microbiome structure/functional changes and validated surrogate markers of host function, host physiology/function, and health. As suggested by Fischbach (27), this research agenda should focus on establishing causation and molecular mechanisms, with an emphasis on phenotypes that are large in magnitude, easy to measure, and unambiguously driven by the microbiota. If such studies are conducted in an equivalent way to studies that established the validity of clinical markers of human disease,

it might be possible to determine what constitutes a healthy microbiome in the future.

Future Considerations

At present, a mechanistic understanding of the host-microbiome relationship is lacking, and there is a need for more research (Table 4).

To determine what constitutes a healthy microbiome, research needs to be conducted with repeated measures within the same individuals to establish mechanistic links between specific microbiome features (diversity, specific taxa, gene clusters, metabolites, etc.) and either function or biomarker or surrogate end point, equivalent to the establishment of LDL cholesterol for cardiovascular disease, glycosylated hemoglobin for diabetes mellitus, or blood pressure for cardiovascular disease. Because of high interindividual variability of the human microbiome, it will be extremely complicated and might even be impossible to identify and validate features of the human microbiome and normal ranges that can be used to predict human health or disease risk.

With the establishment of “microbiome features” that correlate with host function, biomarker, or surrogate end point, it will be possible to devise microbiome-targeted strategies to modulate host indicators of health and possibly decrease disease risk. Just as statins have been tested for efficacy in reducing elevated cholesterol levels to reduce cardiovascular disease risk (165), it should be possible to test efficacy of interventions with probiotics, prebiotics, synbiotics (a combination of probiotics and prebiotics in the same food product), dietary fiber, and FMTs on microbiome features and human health. This will require placebo-controlled human clinical trials in an appropriate target population. Such trials should be performed using an ecological and evolutionary perspective (116) and adequately powered to account for interindividual variation and allow for analysis of subpopulations. Also, given the immense individuality of the gut microbiome, efforts should determine the value of personalizing microbiome-targeted approaches.

Microbiomes are assembled by ecological processes that are complex and often stochastic, and most important, the microbial traits that determine how microbiomes assemble are likely not the same traits that determine health.

Health effects of specific microbiome features might well be context dependent, and bacterial taxa being associated with health in one disease setting and disease in another have already been reported. *Akkermansia*, for example, is correlated with healthier metabolic features in obese individuals (166, 167) and inversely with prevalence of multiple sclerosis (168, 169). It might therefore be impossible to determine microbiome

features that are universally healthy; what constitutes a “healthy microbiome” for one person or human population might well be unhealthy in a different context. For example, a microbiome that contributes to weight gain would be detrimental in an obesogenic environment but would be beneficial during food deprivation. As reviewed above, other factors, such as birth route, antibiotic use, and first diet (breast milk, formula feeding, etc.), may also reduce microbiome diversity, induce detrimental metabolic processes by the microbiota, and contribute to noncommunicable disease risk. It is possible that a progressive deterioration of the human microbiota, attributable to an industrialized lifestyle, is associated with a societywide dysbiosis that is not confined to only a few individuals (170), making it very difficult to characterize features specific to a “healthy gut microbiome.” Rather than trying to define a “healthy” microbiome, future research efforts should determine environmental, clinical, and nutritional factors that decrease symbiotic attributes of the gut microbiome in different societal contexts.

In conclusion, microbiome communities are known to be highly individualized, tend to be stable for years, and show a high degree of interindividual variation. Redundancy in microbiome features is common, and diversity is likely more important than the presence of specific taxa. Ecosystem functions are probably more important than specific individual members (which may even show functional redundancy). As efforts continue to define a “healthy microbiome,” it may be helpful to paraphrase the words of Curtis Huttenhower: “In a forest, a single tree species needn’t be present for a forest to be considered ‘healthy’ (even if many do). The presence of every tree species isn’t a requirement for a forest to be ‘healthy’. Not every ‘healthy’ forest occurs in a typical forest environment (even if many do).”

Acknowledgments

This article is based on information presented and ideas discussed at the North American branch of the ILSI North America’s conference, “Can a Healthy Gut Microbiome Be Defined through Quantifiable Characteristics?” held December 17, 2018, in Washington, DC.

This work was supported by ILSI North America. ILSI North America is a public, nonprofit foundation that provides a forum to advance understanding of scientific issues related to the nutritional quality and safety of the food supply by sponsoring research programs, educational seminars and workshops, and publications. ILSI North America receives support primarily from its industry membership.

The authors’ contributions were as follows—All authors contributed citations and content to the manuscript. All authors read and approved the final version of the manuscript.

References

- Leiby JS, McCormick K, Sherrill-Mix S, Clarke EL, Kessler LR, Taylor LJ, Hofstaedter CE, Roche AM, Mattei LM, Bittinger K, et al. Lack of detection of a human placenta microbiome in samples from preterm and term deliveries. *Microbiome* 2018;6:196.
- Perez-Muñoz ME, Arrieta M-C, Ramer-Tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. *Microbiome* 2017;5:48.
- Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. *Microbiome* 2018;6(1):87.
- Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, Armanini F, Truong DT, Manara S, Zolfo M, et al. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe* 2018;24:133–45.e5.
- Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, de La Cochetiere M-F. Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol* 2013;21:167–73.
- Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci* 2011;108:4578–85.
- Flaherman VJ, Narayan NR, Hartigan-O’Connor D, Cabana MD, McCulloch CE, Paul IM. The effect of early limited formula on breastfeeding, readmission, and intestinal microbiota: a randomized clinical trial. *J Pediatr* 2018;196:84–90.e1.
- Azad SJ, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyskyj AL. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Can Med Assoc J* 2013;185:385–94.
- Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, Björkstén B, Engstrand L, Andersson AF. Decreased gut microbiota diversity, delayed *Bacteroidetes* colonisation and reduced Th1 responses in infants delivered by Caesarean section. *Gut* 2014;63:559–66.
- Ho NT, Li F, Lee-Sarwar KA, Tun HM, Brown BP, Pannaraj PS, Bender JM, Azad MB, Thompson AL, Weiss ST, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun* 2018;9:4169.
- Forbes JD, Azad MB, Vehling L, Tun HM, Konya TB, Guttman DS, Field CJ, Lefebvre D, Sears MR, Becker AB, et al. Association of exposure to formula in the hospital and subsequent infant feeding practices with gut microbiota and risk of overweight in the first year of life. *JAMA Pediatr* 2018;172:e181161.
- Borewicz K, Suarez-Diez M, Hechler C, Beijers R, de Weerth C, Arts I, Penders J, Thijs C, Nauta A, Lindner C, et al. The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life. *Sci Rep* 2019;9:2434.
- McFarland LV, Evans CT, Goldstein EJC. Strain-specificity and disease-specificity of probiotic efficacy: a systematic review and meta-analysis. *Front Med* 2018;5:124.
- Esaiassen E, Hjerde E, Cavanagh JP, Pedersen T, Andresen JH, Rettedal SI, Støen R, Nakstad B, Willassen NP, Klingenberg C. Effects of probiotic supplementation on the gut microbiota and antibiotic resistance development in preterm infants. *Front Pediatr* 2018;6:347.
- Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Med* 2016;8:39.
- Hermansson H, Kumar H, Collado MC, Salminen S, Isolauri E, Rautava S. Breast milk microbiota is shaped by mode of delivery and intrapartum antibiotic exposure. *Front Nutr* 2019;6:4.
- Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. *Trends Mol Med* 2015;21:109–17.
- Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* 2012;489:220–30.
- Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, Young V, Finlay BB. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* 2012;12:611–22.
- Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. *Nature* 2017;548:43–51.
- Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, Breteler MM, Bautista LE, Sharma P, Whitaker JC, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet North Am Ed* 2011;378:584–94.
- Holmes E, Li JV, Marchesi JR, Nicholson JK. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metabolism* 2012;16:559–64.
- Fei N, Zhao L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J* 2013;7:880–4.

24. Surana NK, Kasper DL. Moving beyond microbiome-wide associations to causal microbe identification. *Nature* 2017;552:244–7.
25. Round JL, Palm NW. Causal effects of the microbiota on immune-mediated diseases. *Sci Immunol* 2018;3:eaa01603.
26. Tremlett H, Bauer KC, Appel-Cresswell S, Finlay BB, Waubant E. The gut microbiome in human neurological disease: a review: gut microbiome. *Ann Neurol* 2017;81:369–82.
27. Fischbach MA. Microbiome: focus on causation and mechanism. *Cell* 2018;174:785–90.
28. Martin R, Makino H, Cetinyurek Yavuz A, Ben-Amor K, Roelofs M, Ishikawa E, Kubota H, Swinkels S, Sakai T, Oishi K, et al. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PLoS One* 2016;11:e0158498.
29. Gregory KE, Samuel BS, Houghteling P, Shan G, Ausubel FM, Sadreyev RI, Walker WA. Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. *Microbiome* 2016;4:68.
30. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, Miettinen S, Kukkonen K, Savilahti E, Kuitunen M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome* 2018;6:182.
31. Wu P, Gulati M, Kwok CS, Wong CW, Narain A, O'Brien S, Chew-Graham CA, Verma G, Kadam UT, Mamas MA. Preterm delivery and future risk of maternal cardiovascular disease: a systematic review and meta-analysis. *J Am Heart Assoc* 2018;7:e007809.
32. Hildebrand H, Malmberg P, Askling J, Ekblom A, Montgomery SM. Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. *Scand J Gastroenterol* 2008;43:961–6.
33. Yassour M, Vatanen T, Siljander H, Hämäläinen A-M, Härkönen T, Ryhänen SJ, Franzosa EA, Vlamakis H, Huttenhower C, Gevers D, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med* 2016;8:343ra81.
34. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med* 2016;8(1):51.
35. Lohner S, Küllenberg D, Antes G, Decsi T, Meerpohl JJ. Prebiotics in healthy infants and children for prevention of acute infectious diseases: a systematic review and meta-analysis. *Nutr Rev* 2014;72:523–31.
36. Vellend M. Conceptual synthesis in community ecology. *Q Rev Biol* 2010;85:183–206.
37. Sprockett D, Fukami T, Relman DA. Role of priority effects in the early-life assembly of the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2018;15:197–205.
38. Martinez I, Maldonado-Gomez MX, Gomes-Neto JC, Kittana H, Ding H, Schmaltz R, Joglekar P, Jimenez Cardona R, Marsteller NL, Kembel SW, et al. Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly. *eLife* 2018;7:1–26.
39. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018;555:210–5.
40. Wang J, Thingholm LB, Skieceviciene J, Rausch P, Kummen M, Hov JR, Degenhardt F, Heinsen F-A, Rühlemann MC, Szymczak S, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet* 2016;48:1396–406.
41. Vatanen T, Plichta DR, Somani J, Münch PC, Arthur TD, Hall AB, Rudolf S, Oakeley EJ, Ke X, Young RA, et al. Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life. *Nat Microbiol* 2018;4(3):470–9.
42. Marcobal A, Sonnenburg JL. Human milk oligosaccharide consumption by intestinal microbiota. *Clin Microbiol Infect* 2012;18:12–5.
43. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* 2009;77:2367–75.
44. Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, Knights D, Gajer P, Ravel J, Fierer N, et al. Moving pictures of the human microbiome. *Genome Biol* 2011;12:R50.
45. Gupta VK, Paul S, Dutta C. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Front Microbiol* 2017;8:1162.
46. He Y, Wu W, Zheng H-M, Li P, McDonald D, Sheng H-F, Chen M-X, Chen Z-H, Ji G-Y, Zheng Z-D-X, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med* 2018;24:1532–5.
47. Fedacko J, Takahashi T, Singh RB, Pella D, Chibisov S, Hristova K, Pella D, Elkilany GN, Tomar RS, Juneja LR. Globalization of diets and risk of noncommunicable diseases. In: Watson R, Singh R, Takahashi T, editors. *The role of functional food security in global health*. Associated Press, New York City. 2019. p. 87–107.
48. Campbell-Lendrum D, Prüss-Ustün A. Climate change, air pollution and noncommunicable diseases. *Bull World Health Organ* 2019;97:160–1.
49. Fragiadakis GK, Smits SA, Sonnenburg ED, Van Treuren W, Reid G, Knight R, Manjuran A, Chantalucha J, Dominguez-Bello MG, Leach J, et al. Links between environment, diet, and the hunter-gatherer microbiome. *Gut Microbes* 2019;10(2):216–27.
50. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, Li H, Wang R, Tang J, Huang T, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut* 2019;68(8):1414–29, doi:10.1136/gutjnl-2018-317609.
51. Saxelin M, Lassig A, Karjalainen H, Tynkkynen S, Surakka A, Vapaatalo H, Järvenpää S, Korpela R, Mutanen M, Hatakka K. Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese. *Int J Food Microbiol* 2010;144:293–300.
52. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008;6:e280.
53. Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, Duncan G, Johnstone AM, Lobley GE, Wallace RJ, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* 2011;93:1062–72.
54. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–63.
55. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome* 2016;4:33.
56. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One* 2010;5:e15046.
57. Hadizadeh F, Walter S, Belheouane M, Bonfiglio F, Heinsen F-A, Andreasson A, Agreus L, Engstrand L, Baines JF, Rafter J, et al. Stool frequency is associated with gut microbiota composition. *Gut* 2017;66:559–60.
58. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* 2016;65:57–62.
59. Roager HM, Hansen LBS, Bahl MI, Frandsen HL, Carvalho V, Göbel RJ, Dalgaard MD, Plichta DR, Sparholt MH, Vestergaard H, et al. Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* 2016;1(9):16093.
60. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–63.
61. Venkataraman A, Sieber JR, Schmidt AW, Waldron C, Theis KR, Schmidt TM. Variable responses of human microbiomes to dietary supplementation with resistant starch. *Microbiome* 2016;4:33.
62. Martínez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One* 2010;5:e15046.
63. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, Hansen TH, Liang S, Feng Q, Zhang C, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat Microbiol* 2018;3:1255–65.

64. Schlomann BH, Wiles TJ, Wall ES, Guillemin K, Parthasarathy R. Low-dose antibiotics can collapse gut bacterial populations via a gelation transition. *Microbiology*. [Internet]. 2019. Available from: <http://biorxiv.org/lookup/doi/10.1101/565556>.
65. Janssens Y, Nielandt J, Bronselaer A, Debonne N, Verbeke F, Wynendaele E, Van Immerseel F, Vandewynckel Y-P, De Tré G, De Spiegeleer B. Disbioeme database: linking the microbiome to disease. *BMC Microbiol* 2018;18:50.
66. Insel R, Knip M. Prospects for primary prevention of type 1 diabetes by restoring a disappearing microbe. *Pediatr Diabetes* 2018;19:1400–6.
67. Maldonado-Gómez MX, Martínez I, Bottacini F, O'Callaghan A, Ventura M, van Sinderen D, Hillmann B, Vangay P, Knights D, Hutkins RW, et al. Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on individualized features of the resident microbiome. *Cell Host Microbe* 2016;20:515–26.
68. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, Tremaroli V, Bakker GJ, Attaye I, Pinto-Sietsma S-J, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med* 2018;24:1526–31.
69. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, Lucas SK, Beura LK, Thompson EA, Till LM, et al. US immigration westernizes the human gut microbiome. *Cell* 2018;175:962–72.e10.
70. Miller GE, Engen PA, Gillevet PM, Shaikh M, Sikaroodi M, Forsyth CB, Mutlu E, Keshavarzian A. Lower neighborhood socioeconomic status associated with reduced diversity of the colonic microbiota in healthy adults. *PLoS One* 2016;11:e0148952.
71. Snijder MB, Galenkamp H, Prins M, Derks EM, Peters RJG, Zwinderman AH, Stronks K. Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open* 2017;7:e017873.
72. Beukers MH, Dekker LH, de Boer EJ, Perenboom CWM, Meijboom S, Nicolaou M, de Vries JHM, Brants HAM. Development of the HELIUS food frequency questionnaires: ethnic-specific questionnaires to assess the diet of a multiethnic population in The Netherlands. *Eur J Clin Nutr* 2015;69:579–84.
73. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen M, Hercog R, Jung F-E, et al. Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol* 2017;35(11):1069–76.
74. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–14.
75. Kritchevsky D. Dietary fiber. *Ann Rev Nutr* 1988;8:301–28.
76. Trowell HC, Burkitt DP. The development of the concept of dietary fibre. *Mol Aspects Med* 1987;9:7–15.
77. Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. *Nutr J* 2014;13:34.
78. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe* 2018;23:705–15.
79. Flint HJ, Duncan SH, Louis P. The impact of nutrition on intestinal bacterial communities. *Curr Opin Microbiol* 2017;38:59–65.
80. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metabolism* 2014;20:779–86.
81. McBurney MI, Reimer RA, Tappenden KA. Short chain fatty acids, intestinal adaptation, and nutrient utilization. *Adv Exp Med Biol* 1997;427:135–43.
82. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016;167:1339–53.e21.
83. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, Amieva MR, Huang KC, Sonnenburg JL. Quantitative imaging of gut microbiota spatial organization. *Cell Host Microbe* 2015;18:478–88.
84. Windey K, De Preter V, Louat T, Schuit F, Herman J, Vansant G, Verbeke K. Modulation of protein fermentation does not affect fecal water toxicity: a randomized cross-over study in healthy subjects. *PLoS One* 2012;7:e52387.
85. Windey K, François I, Broekaert W, De Preter V, Delcour JA, Louat T, Herman J, Verbeke K. High dose of prebiotics reduces fecal water cytotoxicity in healthy subjects. *Mol Nutr Food Res* 2014;58:2206–18.
86. McBurney MI, Soest PJV, Jeraci JL. Colonic carcinogenesis: The microbial feast or famine mechanism. *Nutr Cancer* 1987;10:23–8.
87. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* 1991;70:443–59.
88. Windey K, De Preter V, Huys G, Broekaert WF, Delcour JA, Louat T, Herman J, Verbeke K. Wheat bran extract alters colonic fermentation and microbial composition, but does not affect faecal water toxicity: a randomised controlled trial in healthy subjects. *Br J Nutr* 2015;113:225–38.
89. François IEJA, Lescroart O, Ververbeke WS, Marzorati M, Possemiers S, Evenepoel P, Hamer H, Houben E, Windey K, Welling GW, et al. Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: a double-blind, randomised, placebo-controlled, cross-over trial. *Br J Nutr* 2012;108:2229–42.
90. De Preter V, Coopmans T, Rutgeerts P, Verbeke K. Influence of long-term administration of lactulose and *Saccharomyces boulardii* on the colonic generation of phenolic compounds in healthy human subjects. *J Am Coll Nutr* 2006;25:541–9.
91. La Fata G, Rastall RA, Lacroix C, Harmsen HJM, Mohajeri MH, Weber P, Steinert RE. Recent development of prebiotic research—statement from an expert workshop. *Nutrients* 2017;9:1376.
92. Gibson G, Hutkins R, Sanders M, Prescott S, Reimer R, Salminen S, Scott K, Stanton C, Swanson K, Cani P, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017;14:491–502.
93. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 2015;12:303–10.
94. Krumbeck JA, Rasmussen HE, Hutkins RW, Clarke J, Shawron K, Keshavarzian A, Walter J. Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome* 2018;6(1):121.
95. Buddington R, Kapadia C, Neumer F, Theis S. Oligofructose provides laxation for irregularity associated with low fiber intake. *Nutrients* 2017;9:1372.
96. Schroeder BO, Birchenough GMH, Ståhlman M, Arike L, Johansson MEV, Hansson GC, Bäckhed F. *Bifidobacteria* or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell Host Microbe* 2018;23:27–40. e7.
97. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. *Gut Microbes* 2017;8:172–84.
98. Carlson J, Erickson J, Hess J, Gould T, Slavin J. Prebiotic dietary fiber and gut health: comparing the in vitro fermentations of beta-glucan, inulin and xylooligosaccharide. *Nutrients* 2017;9:1361.
99. Watson D, O'Connell Motherway M, Schoterman MH, van Neerven RJJ, Nauta A, van Sinderen D. Selective carbohydrate utilization by lactobacilli and bifidobacteria. *J Appl Microbiol* 2013;114:1132–46.
100. McBurney MI. Potential water-holding capacity and short-chain fatty acid production from purified fiber sources in a fecal incubation system. *Nutrition* 1991;7:421–4.
101. François IEJA, Lescroart O, Ververbeke WS, Marzorati M, Possemiers S, Hamer H, Windey K, Welling GW, Delcour JA, Courtin CM, et al. Effects of wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal parameters in healthy preadolescent children. *J Pediatr Gastroenterol Nutr* 2014;58:647–53.
102. Damen B, Cloetens L, Broekaert WF, François I, Lescroart O, Trogh I, Arnaut F, Welling GW, Wijffels J, Delcour JA, et al. Consumption of breads containing in situ-produced arabinoxylan oligosaccharides alters gastrointestinal effects in healthy volunteers—3. *J Nutr* 2012;142:470–7.
103. Davis LMG, Martínez I, Walter J, Goin C, Hutkins RW. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One* 2011;6:e25200.
104. Lam YY, Zhang C, Zhao L. Causality in dietary interventions—building a case for gut microbiota. *Genome Med* 2018;10:62.

105. François IEJA, Lescroart O, Veraverbeke WS, Marzorati M, Possemiers S, Hamer H, Windey K, Welling GW, Delcour JA, Courtin CM, et al. Effects of wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal parameters in healthy preadolescent children. *J Pediatr Gastroenterol Nutr* 2014;58:647–53.
106. Bindels LB, Segura Munoz RR, Gomes-Neto JC, Mutemberezi V, Martínez I, Salazar N, Cody EA, Quintero-Villegas MI, Kittana H, de los Reyes-Gavilán CG, et al. Resistant starch can improve insulin sensitivity independently of the gut microbiota. *Microbiome* 2017;5(1):12.
107. Martínez I, Muller CE, Walter J. Long-term temporal analysis of the human fecal microbiota revealed a stable core of dominant bacterial species. *PLoS One* 2013;8:e69621.
108. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, et al. Genomic variation landscape of the human gut microbiome. *Nature* 2012;493:45–50.
109. Lahti L, Salonen A, Kekkonen RA, Salojärvi J, Jalanka-Tuovinen J, Palva A, Orešič M, de Vos WM. Associations between the human intestinal microbiota, *Lactobacillus rhamnosus* GG and serum lipids indicated by integrated analysis of high-throughput profiling data. *PeerJ* 2013;1:e32.
110. Kristensen NB, Bryrup T, Allin KH, Nielsen T, Hansen TH, Pedersen O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Med* 2016;8(1):52.
111. Taverniti V, Koirala R, Dalla Via A, Gargari G, Leonardi E, Arioli S, Guglielmetti S. Effect of cell concentration on the persistence in the human intestine of four probiotic strains administered through a multispecies formulation. *Nutrients* 2019;11:285.
112. Jacobsen CN, Nielsen VR, Hayford AE, Møller PL, Michaelsen KF, Tvede M, Jakobsen M. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. *Appl Environ Microbiol* 1999;65:8.
113. Tannock GW, Munro K, Harmsen HJM, Welling GW, Smart J, Gopal PK. Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. *Appl Environ Microbiol* 2000;66:2578–88.
114. Rattanaprasert M, Roos S, Hutkins RW, Walter J. Quantitative evaluation of synbiotic strategies to improve persistence and metabolic activity of *Lactobacillus reuteri* DSM 17938 in the human gastrointestinal tract. *J Funct Foods* 2014;10:85–94.
115. Davoren MJ, Liu J, Castellanos J, Rodríguez-Malavé NI, Schiestl RH. A novel probiotic, *Lactobacillus johnsonii* 456, resists acid and can persist in the human gut beyond the initial ingestion period. *Gut Microbes* 2018; Dec 22, doi: 10.1080/19490976.2018.1547612. [Epub ahead of print].
116. Walter J, Maldonado-Gómez MX, Martínez I. To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. *Curr Opin Biotechnol* 2018;49:129–39.
117. Lawley TD, Walker AW. Intestinal colonization resistance. *Immunology* 2013;138:1–11.
118. Eloe-Fadrosh EA, Brady A, Crabtree J, Drabek EF, Ma B, Mahurkar A, Ravel J, Haverkamp M, Fiorino A-M, Botelho C, et al. Functional dynamics of the gut microbiome in elderly people during probiotic consumption. *mBio* 2015;6:e00231–15.
119. Rutishauser IH. Dietary intake measurements. *Public Health Nutr* 2005;8(7A):1100–7.
120. Beaton GH, Milner J, McGuire V, Feather TE, Little JA. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. *Am J Clin Nutr* 1983;37:986–95.
121. Song SJ, Amir A, Metcalf JL, Amato KR, Xu ZZ, Humphrey G, Knight R. Preservation methods differ in fecal microbiome stability, affecting suitability for field studies. *mSystems* 2016;1(3):e00021–16.
122. Voigt AY, Costea PI, Kultima JR, Li SS, Zeller G, Sunagawa S, Bork P. Temporal and technical variability of human gut metagenomes. *Genome Biol* 2015;16:73.
123. von Wintzingerode F, Göbel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* 1997;21:213–29.
124. Cammarota G, Ianaro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, Sokol H, Arkkila P, Pintus C, Hart A, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 2017;66:569–80.
125. Mould FL, Kliem KE, Morgan R, Mauricio RM. In vitro microbial inoculum: a review of its function and properties. *Anim Feed Sci Technol* 2005;123–124:31–50.
126. Wedekind KJ, Mansfield HR. Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. *Appl Environ Microbiol* 1988;54:6.
127. Kennedy NA, Walker AW, Berry SH, Duncan SH, Farquarson FM, Louis P, Thomson JM, Satsangi J, Flint HJUK IBD Genetics Consortium, et al., UK IBD Genetics Consortium The impact of different DNA extraction kits and laboratories upon the assessment of human gut microbiota composition by 16S rRNA gene sequencing. *PLoS One* 2014;9:e88982.
128. FAO and WHO. Codex alimentarius: guidelines on nutrition labeling (CAC/GL 2–1995). [Internet]. 2017. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%2F53A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BGL%2B2-1985%252FCXG_002e.pdf.
129. FAO and WHO. Codex alimentarius: general guidelines on claims (CAC/GL 1–1979). [Internet]. 1979. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BGL%2B1-1979%252FCXG_001e.pdf.
130. FDA. Federal Food, Drug, and Cosmetic Act, as amended. [Internet]. 1938 [cited 2019 Apr 11]. Available from: <https://www.fda.gov/regulatoryinformation/lawsenforcedbyfda/federalfooddrugandcosmeticactfdca/default.htm>.
131. FDA. Final rule: food labeling: requirement for nutrient content claims, health claims, and statements of nutritional support for dietary supplements. *Fed Regist* 2014;62:49863–6.
132. Health Canada. Nutrition labelling. [Internet]. Nutrition Labeling 2015 [cited 2019 Mar 11]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/nutrition-labelling.html>.
133. Health Canada. Health claims. [Internet]. 2016 [cited 2019 Mar 7]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/health-claims.html>.
134. Government of Canada. Food and Drugs Act. [Internet]. 2018 [cited 2019 Apr 11]. Available from: <https://laws-lois.justice.gc.ca/eng/acts/F-27/index.html>.
135. European Commission. Regulation (EC) No 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on food. *Official Journal of the European Union* 2006;L404:9–25, Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2006:404:TOC>.
136. FDA. Food Labeling: nutrient content claims; alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid omega-3 fatty acids. *Fed Regist* 2014;79:23262–73.
137. FDA. Notice of availability: the declaration of certain isolated or synthetic non-digestible carbohydrates as dietary fiber on nutrition and supplement facts labels; guidance for industry; availability. *Fed Regist* 2018;83:27894–5.
138. Health Canada. List of dietary fibres reviewed and accepted by Health Canada's Food Directorate. [Internet]. 2017 [cited 2019 Mar 12]. Available from: <https://www.canada.ca/en/health-canada/services/publications/food-nutrition/list-reviewed-accepted-dietary-fibres.html>.
139. FDA. Examples of physiological effects that are beneficial to human health. [Internet]. Questions and Answers on Dietary Fiber 2016 [cited 2019 Mar 12]. Available from: https://www.fda.gov/food/labelingnutrition/ucm528582.htm#beneficial_physiological_effects.
140. Health Canada. 4.0 Physiological effects. [Internet]. Policy for labelling and advertising of dietary fibre-containing food products. 2017 [cited 2019 Mar 12]. Available from: <https://www.canada.ca/en/health-canada/services/publications/food-nutrition/labelling-advertising-dietary-fibre-food-products.html#a4>.
141. European Commission. Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and healthText with EEA relevance. *Official Journal of the European Union* 2012;L136:1–40,

- Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2012:136:TOC>.
142. FDA. Authorized health claims that meet the significant scientific agreement (SSA) standard. [Internet]. Labeling & Nutrition 2018 [cited 2019 Mar 7]. Available from: <https://www.fda.gov/food/labeling-nutrition/ucm2006876.htm#approved>.
 143. EFSA. Claims on disease risk reduction and child development or health. [Internet]. 2017 [cited 2019 Mar 7]. Available from: <https://www.efsa.europa.eu/en/topics/topic/claims-disease-risk-reduction-and-child-development-or-health-under-article-14>.
 144. Health Canada. Health claims reviewed: accepted and not accepted. [Internet]. Health Claim Assessments. 2017 [cited 2019 Mar 7]. Available from: <https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/health-claims/assessments.html>.
 145. FDA. Food labeling: health claims; oats and coronary heart disease. Fed Regist 1997;62:3584–99.
 146. Health Canada. Accepted claims about the nature of probiotic microorganisms in food. [Internet]. AEM 2009 [cited 2018 Nov 21]. Available from: https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/health-claims/accepted-claims-about-nature-probiotic-microorganisms-food.html#fn_t1b1.
 147. FDA. Guidance for industry: evidence-based review system for the scientific evaluation of health claims. [Internet]. Guidance Documents & Regulatory Information. 2009 [cited 2019 Mar 7]. Available from: <https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm073332.htm>.
 148. EFSA. Outcome of a public consultation on the discussion paper for the revision of the guidance on the scientific requirements for health claims related to gut and immune function. EFSA J 2015;12:1–117.
 149. NDAC. Regulation of the National Agency for Drug and Food Control of the Republic of Indonesia concerning monitoring of claims on processed food label and advertising, National Agency for Drug and Food Control of the Republic of Indonesia, Jakarta. 2016.
 150. Tanemura N, Hamadate N, Urushihara H. Evaluation of randomized controlled trials of foods with functional claims request: the learning outcomes from studies in Japan. J Funct Foods 2018;42:248–53.
 151. Japan Ministry of Health, Labour, and Welfare. Food with health claims, food for special dietary uses, and nutrition labeling. [Internet]. Foods for Specified Health Uses (FOSHU). 1991 [cited 2019 Mar 11]. Available from: <https://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html>.
 152. Japan Consumer Affairs Agency. Foods for specified health permission (approved) item list. [Internet]. 2018 [cited 2019 Mar 11]. Available from: https://www.caa.go.jp/policies/policy/food_labeling/health_promotion/#m02-1.
 153. MOH Malaysia. Nutrition labelling. [Internet]. Ministry of Health, Malaysia. [Internet]. 1998. Available from: <http://fsq.moh.gov.my>.
 154. MOH Malaysia. Guide to nutrition labelling and claims. [Internet]. Food Safety and Quality Division, Ministry of Health Malaysia; 2010. Available from: <http://fsq.moh.gov.my>.
 155. AVA, Singapore. A guide to food labelling and advertisements. [Internet]. Agri-Food & Veterinary Authority, Singapore; 2016. Available from: <http://www.ava.gov.sg/>.
 156. MOPH Thailand. Nutrition labelling. [Internet]. Ministry of Health, Thailand; 1998. Available from: <http://fsq.moh.gov.my>.
 157. Japan Ministry of Health, Labour, and Welfare. Food with health claims, food for special dietary uses, and nutrition labeling. [Internet]. 1991 [cited 2019 Mar 11]. Available from: <https://www.mhlw.go.jp/english/topics/foodsafety/fhc/>.
 158. Japan Consumer Affairs Agency. Guidance for industry: the system of “foods with function claims” has been launched! [Internet]. Japan Consumer Affairs Agency; 2015 [cited 2019 Mar 11]. Available from: https://www.caa.go.jp/policies/policy/food_labeling/information/pamphlets/pdf/151224_2.pdf.
 159. Wong CB, Odamaki T, Xiao J. Beneficial effects of Bifidobacterium longum subsp. longum BB536 on human health: modulation of gut microbiome as the principal action. J Funct Foods 2019;54:506–19.
 160. Brazilian Health Regulatory Agency (ANVISA). Draft guideline to request evaluation of probiotics for use in food. [Internet]. Ministry of Health, Brazil; 2019. Available from: http://portal.anvisa.gov.br/documents/3845226/0/Guia+Probioticos_Portal.pdf/e1bbf33e-719e-4f3e-84a0-7846bbe17972.
 161. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) resource. [Internet]. 2016 [cited 2019 Mar 11]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK326791/>.
 162. Robb MA, McInnes PM, Califf RM. Biomarkers and surrogate endpoints: developing common terminology and definitions. JAMA 2016;315:1107–8.
 163. Sundin J, Öhman L, Simrén M. Understanding the gut microbiota in inflammatory and functional gastrointestinal diseases. Psychosom Med 2017;79:857–67.
 164. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, Kurilshikov A, Bonder MJ, Valles-Colomer M, Vandeputte D, et al. Population-level analysis of gut microbiome variation. Science 2016;352:560–4.
 165. Armitage J, Baigent C, Barnes E, Betteridge DJ, Blackwell L, Blazing M, Bowman L, Braunwald E, Byington R, Cannon C, et al. Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials. Lancet North Am Ed 2019;393:407–15.
 166. Dao MC, Everard A, Aron-Wisnewsky J, Sokolowska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyle L, et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut 2016;65:426–36.
 167. Schneeberger M, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, Gomis R, Claret M, Cani PD. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. Sci Rep 2015;5:16643.
 168. Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, Venkatesan A, Fraser CM, Mowry EM. Gut microbiota in multiple sclerosis. J Investig Med 2015;63:729.
 169. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Liu S, Glanz BL, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun 2016;7:12015.
 170. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications for human health. Nat Rev Microbiol 2019;17:383–90.